CLAIMS

1. Metazoan organism, with the exception of humans, characterized in that at least one cell of said organism comprises at least:

(i) one fusion protein comprising sequentially:
- a recombinase protein;
- a hinge region of at least 15 amino acids;
- a polypeptide comprising the ligand-binding domain of the human nuclear estrogen receptor, or of a vertebrate nuclear estrogen receptor, and their natural variants or one of their fragments, said polypeptide exhibiting at least one mutation relative to the wild-type form of said ligand-binding domains, or of their natural variants, or of their fragments,

said fusion protein having a negligible, or even zero, recombinase activity in the presence of a natural ligand and a recombinase activity induced by small quantities of synthetic ligand endowed with antiestrogenic activity;

(ii) one or more gene or intergenic DNA sequences of interest naturally belonging to said genome of said organism into which one or more recognition sites of said recombinase protein are inserted, said DNA sequence(s) of interest being located in one or more of the chromosomes of the genome of said cell.

2. Organism according to Claim 1, characterized in that said recombinase protein is preferably selected from the group of site-specific recombinases composed of the Cre recombinase of bacteriophage P1, the FLP recombinase of *Saccharomyces cerevisiae*, the R recombinase of *Zygosaccharomyces rouxii* pSR1, the A recombinase of *Kluyveromyces drosophilarium* pKD1, the A recombinase of *Kluyveromyces waltii* pKW1, the integrase λ Int, the recombinase of the GIN recombination system of the Mu phage, of the bacterial β recombinase or a variant thereof.

3. Organism according to Claim 2, characterized in that the recombinase is the Cre recombinase of bacteriophage P1 and its natural or synthetic variants.

4. Organism according to Claims 1 to 3, characterized in that said sites of recognition specific for said Cre recombinase are preferably chosen from the group composed of the sequences Lox P, Lox 66, Lox 71, Lox 511, Lox 512, Lox 514.

5. Organism according to Claims 1 to 4, characterized in that said hinge region comprises all or part of the D hinge region of a nuclear estrogen receptor, a region situated between the DNA-binding domain and the ligand-binding domain, or a peptide which is functionally equivalent to said D hinge region.

6. Organism according to Claim 5, characterized in that said hinge region comprises amino acids 282 to 301 of the sequence SEQ ID No. 2.

7. Organism according to one of the preceding claims, characterized in that said polypeptide chosen from the ligand-binding domain of the nuclear human estrogen receptors or its natural variants, or one of their fragments, is the ligand-binding domain of the human nuclear estrogen receptor α or one of its natural variants, or one of their fragments and in that said ligand-binding domain or one of its natural variants, or one of their fragments exhibits at least one mutation chosen from the group:

- mutation (G521R) glycine to arginine at position 521 of the sequence SEQ ID No. 2 or of a natural variant of this sequence;
- mutation (G400V) glycine to valine at position 400 of the sequence SEQ ID No. 2 or of a natural variant of this sequence;
- mutation (methionine-leucine) to (alanine-alanine) situated at position 543-544 (M543A/L544A mutation) of the sequence SEQ ID No. 2 or of a natural variant of this sequence.

8. Organism according to any one of the preceding claims, characterized in that said fusion protein is encoded by a fusion gene integrated into one or more of the chromosomes of said cell of said organism, said fusion gene being under the control of expression elements ensuring its expression in at least one cell of said organism.

9. Organism according to any one of Claims 1 to 7, characterized in that said fusion protein is encoded by a fusion gene integrated into an extrachromosomal

expression vector, said fusion gene being under the control of expression elements ensuring its expression in at least one cell of said organism.

10. Organism according to either of Claims 8 and 9, characterized in that said expression elements are chosen from elements controlling tissue-specific and cell-specific expression or ubiquitous expression.

11. Organism according to Claims 8 to 10, characterized in that said elements controlling expression are chosen from elements controlling expression ensuring constitutive expression or elements controlling expression ensuring inducible expression.

12. Organism according to Claims 8 to 11, characterized in that said expression element is chosen from the group composed of the promoter regions of cytokeratin 14 (K 14), of cytokeratin 5 (K 5), of the adipocyte fatty acid binding protein 2 (aP2) and of α-1-antitrypsin.

13. Organism according to one of Claims 8 to 12, characterized in that said fusion gene having the sequence SEQ ID No. 3 encodes the fusion protein Cre-ER^{T} having the sequence SEQ ID No. 4.

14. Organism according to any one of Claims 8 to 12, characterized in that said fusion gene having the sequence SEQ ID No. 5 encodes the fusion protein Cre-ER^{T2} having the sequence SEQ ID No. 6.

15. Organism according to any one of Claims 8 to 12, characterized in that said fusion gene having the sequence SEQ ID No. 7 encodes the fusion protein Cre-ER^{T3} having the sequence SEQ ID No. 8.

16. Organism according to Claims 9 to 15, characterized in that said fusion gene preferably comprises in the 5′ → 3′ direction:

- a DNA fragment encoding the Cre recombinase of bacteriophage P1 or one of its variants;
- a DNA fragment of at least 45 nucleotides encoding at least either all or part of the D hinge region of a nuclear estrogen receptor, or a peptide which is functionally equivalent to said D hinge region; and
- a DNA fragment encoding the ligand-binding domain (LBD) of a nuclear estrogen receptor or variants thereof, said DNA fragment having at least one mutation conferring on LBD the capacity to respond to synthetic antiestrogens, but not to natural estrogenic agonists.

17. Organism according to any one of Claims 1 to 7, characterized in that said fusion protein is introduced into at least one cell of said organism.

18. Organism according to any one of Claims 1 to 17, characterized in that said synthetic ligand endowed with antiestrogenic activity inducing the activity of the recombinase is chosen from the group composed of Tamoxifen, 4-hydroxyTamoxifen, ICI 164 384 and ICI 182 780.

19. Organism according to any one of the preceding claims, characterized in that said DNA sequence of interest is a gene selected from the group composed of RXR_α, RXR_β, RXR_γ, RAR_α, RAR_β, RAR_γ, $SNF2_\beta$.

20. Organism according to any one of the preceding claims, characterized in that said organism is an animal, in particular a mouse.

21. Organism according to Claim 20, characterized in that at least one of the cells of said mouse comprises:

- a fusion gene encoding the fusion protein Cre-ER^{T} having the sequence SEQ ID No. 4, or Cre-ER^{T2} having the sequence ID No. 6, or Cre-ER^{T3} having the sequence ID No. 8, said fusion gene being under the control of the cytokeratin K5 promoter region;
- one or more chromosomal DNA sequences of interest in their natural chromatin context and flanked ("floxed") by a lox site.

22. Organism according to Claim 20, characterized in that at least one of the cells of said mouse comprises:

- a fusion gene encoding the fusion protein Cre-ER^{T} having the sequence SEQ ID No. 4, or Cre-ER^{T2} having the sequence ID No. 6, or Cre-ER^{T3} having the sequence ID No. 8, said fusion gene being under the control of the cytokeratin K14 promoter region;
- one or more chromosomal DNA sequences of interest in their natural chromatin context and flanked ("floxed") by a lox site.

23. Organism according to Claim 20, characterized in that at least one of the cells of said mouse comprises:

- a fusion gene encoding the fusion protein Cre-ER^{T} having the sequence SEQ ID No. 4, or Cre-ER^{T2} having the sequence ID No. 6, or Cre-ER^{T3} having the sequence ID No. 8, said fusion gene being under the control of the adipocyte fatty acid binding protein 2 (aP2) promoter region;
- one or more chromosomal DNA sequences of interest in their natural chromatin context and flanked ("floxed") by a lox site.

24. Organism according to Claim 20, characterized in that at least one of the cells of said mouse comprises:

- a fusion gene encoding the fusion protein Cre-ER^{T} having the sequence SEQ ID No. 4, or Cre-ER^{T2} having the sequence ID No. 6, or Cre-ER^{T3} having the sequence ID No. 8, said fusion gene being under the control of the α-1-antitrypsin promoter region;
- one or more chromosomal DNA sequences of interest in their natural chromatin context and flanked ("floxed") by a lox site.

25. Method of preparing a metazoan organism according to any one of the preceding claims, characterized in that it comprises the following steps:

a) obtaining an embryonic stem (ES) cell modified by insertion of site(s) of recognition for said recombinase protein into said DNA sequence(s) of interest, located in one or more chromosomes, by homologous recombination;

b) introducing said modified embryonic stem cell into an embryo of said organism;

c) developing said embryo up to the stage of a fertile adult organism;

d) crossing said fertile adult organism with a transgenic organism in which at least one of the cells expresses said fusion protein and obtaining the progeny derived from said crossing; and

e) optionally, selecting, among said progeny, said metazoan organism.

26. Method of preparing a metazoan organism according to any one of Claims 1 to 24, characterized in that it comprises the following steps:

a) obtaining a somatic cell modified by insertion of site(s) of recognition for said recombinase protein into said DNA sequence(s) of interest, located in one or more chromosomes, by homologous recombination;

b) transferring the nucleus of said modified somatic cell into the cytoplasm of an enucleated recipient oocyte;

c) developing the embryo obtained in step b) up to the stage of a fertile adult organism;

d) crossing said fertile adult organism with a transgenic organism in which at least one of the cells expresses said fusion protein and obtaining the progeny derived from said crossing; and

e) optionally, selecting, among the progeny, said metazoan organism.

27. Method of preparing a metazoan organism according to any one of Claims 1 to 24, characterized in that it comprises the following steps:

a) obtaining an embyronic stem (ES) cell modified by insertion of site(s) of recognition for said recombinase protein into said DNA sequence(s) of interest, located in one or more chromosomes, by homologous recombination;

b) introducing said modified embryonic stem cell into an embryo of said organism;

c) developing said embryo; and

d) introducing said fusion protein into at least one cell of said embryo or of the organism obtained from the development of said embryo.

28. Method of preparing a metazoan organism according to any one of Claims 1 to 24, characterized in that it comprises the following steps:

a) obtaining a somatic cell modified by insertion of site(s) of recognition for said recombinase protein into said DNA sequence(s) of interest, located in one or more chromosomes, by homologous recombination;

b) transferring the nucleus of said modified somatic cell into the cytoplasm of an enucleated recipient oocyte;

c) developing said embryo; and

d) introducing said fusion protein into at least one cell of said embryo or of said organism obtained from the development of said embryo.

29. Method of conditional recombination, in particular excision, insertion, inversion, translocation, at the level of the DNA sequence of interest into which there is (are) inserted one or more sites of recognition for said recombinase protein, said DNA sequence of interest being located in one or more of the chromosomes of said genome of said cell of said organism according to Claims 1 to 24, characterized in that it comprises the steps of:

(i) bringing at least one cell of said organism into contact with a synthetic ligand endowed with antiestrogenic activity;

(ii) inducing the activity of the recombinase of said fusion protein by said synthetic ligand.

30. Method of conditional deletion of a DNA fragment in which a method of excision according to Claim 29 is used and in which said DNA fragment(s) to be excised is (are) flanked by two recombinase protein recognition sites oriented as a direct repeat.

31. Method of obtaining a metazoan organism, with the exception of humans, in which at least one cell possesses an allele of a gene of interest inactivated by a method of conditional deletion and in which the other allele of said gene of interest possesses a mutation, preferably limited, in exon and/or regulatory sequences, said method being characterized in that it makes it possible to obtain, in a metazoan organism, somatic mutations controlled in space and time, and which are limited (point mutations, small deletions or

insertion) in exon and/or regulatory sequences, and in that it comprises the steps of:

a) obtaining a metazoan organism in which at least one cell of the germ line comprises said mutation in one of the alleles of said gene of interest;

b) crossing said organism obtained in step a) with an organism according to any one of Claims 1 to 24;

c) selecting a progeny whose genome comprises a gene of interest in which one of the alleles possesses a mutation and the other allele possesses at least two recombinase protein recognition sites oriented as a direct repeat;

d) using the method according to Claims 29 or 30 of conditional deletion, of the DNA fragment of said allele of said gene of interest which is flanked by at least two recombinase protein recognition sites oriented as a direct repeat; and

e) obtaining said metazoan organism in which the genome of at least one cell comprises said gene of interest in which one allele is inactivated, while the other allele possesses a somatic, preferably limited, mutation and preferably in exon and/or regulatory sequences.

32. Method according to any one of Claims 29 to 31, characterized in that said sites of recognition specific for the recombinase protein are Lox P sites and said recombinase protein is the Cre protein of bacteriophage P1, or one of its variants.

33. Organism capable of being obtained using a method according to Claims 25 to 32.

34. Organism according to Claim 33, characterized in that it is an animal, in particular a mouse.

35. Method of analyzing or studying the biological function of a DNA sequence of interest, in particular of a gene, characterized in that it comprises the steps of:

(i) bringing an organism according to one of Claims 1 to 24, 33 and 34 or cells isolated from said organism into contact with a synthetic ligand endowed with antiestrogenic activity;

(ii) optionally inducing the expression of said fusion protein;

(iii) revealing the recombination event catalyzed by the recombinase activity of said fusion protein;

(iv) biochemical and/or physiological and/or phenotypic and/or behavioral study or analysis of said cell or of said organism.

36. Method according to Claims 29, 30, 31, 32 and 35, characterized in that the bringing of said cells of said organism into contact with said synthetic ligand is carried out according to a route of administration chosen from the oral route, the topical route, injection, in particular intramuscular, intravenous, intracerebral, intraspinal and intraperitoneal injection, or in the case of embryos, fetuses and neonates before weaning by administering said synthetic ligand to the mother.

37. Method of screening compounds capable of being used as a medicament for the preventive and/or curative

treatment of pathological conditions associated with alteration of the expression and/or of the function of said DNA sequence of interest, characterized in that it comprises the step of administering said compound to an organism according to claims 1 to 24, 33 and 34.

38. Use of an organism according to any one of Claims 1 to 24, 33 and 34 or of cells derived from said organism for carrying out a spatiotemporally controlled site-specific recombination of said DNA sequence of interest in its natural chromatin environment, with an efficiency of at least 85%, in the presence of synthetic ligand endowed with antiestrogenic activity in the cells of said organism expressing said fusion protein, and with an efficiency at least lower than 5%, in the absence of synthetic ligand or in the presence of a natural estrogen in the cells of said organism expressing said fusion protein.

39. Use according to Claim 38, characterized in that said cells of said organism are chosen from the cells of the epidermis, the hepatocytes and the adipocytes.

40. Transgenic mouse K5-Cre-ER^T/$RXR\alpha^{L2/L2}$ whose $RXR\alpha$ gene may be selectively inactivated in the basal keratinocytes of the epidermis using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alopecia and/or hyperproliferation of the basal keratinocytes and/or an inflammatory reaction of the skin.

41. Transgenic mouse K5-Cre-ER^{T2}/RXR$\alpha^{L2/L2}$ whose RXRα gene may be selectively inactivated in the basal keratinocytes of the epidermis using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alopecia and/or hyperproliferation of the basal keratinocytes and/or an inflammatory reaction of the skin.

42. Transgenic mouse K5-Cre-ER^{T3}/RXR$\alpha^{L2/L2}$ whose RXRα gene may be selectively inactivated in the basal keratinocytes of the epidermis using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alopecia and/or hyperproliferation of the basal keratinocytes and/or an inflammatory reaction of the skin.

43. Transgenic mouse K14-Cre-ER^{T}/RXR$\alpha^{L2/L2}$ whose RXRα gene may be selectively inactivated in the basal keratinocytes of the epidermis using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alopecia and/or hyperproliferation of the basal keratinocytes and/or an inflammatory reaction of the skin.

44. Transgenic mouse K14-Cre-ER^{T2}/RXR$\alpha^{L2/L2}$ whose RXRα gene may be selectively inactivated in the basal keratinocytes of the epidermis using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alopecia and/or hyperproliferation of the

basal keratinocytes and/or an inflammatory reaction of the skin.

45. Transgenic mouse K14-Cre-ER^{T3}/$RXR\alpha^{L2/L2}$ whose $RXR\alpha$ gene may be selectively inactivated in the basal keratinocytes of the epidermis using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alopecia and/or hyperproliferation of the basal keratinocytes and/or an inflammatory reaction of the skin.

46. Transgenic mouse αAT-Cre-ER^{T}/$RXR\alpha^{L2/L2}$ whose $RXR\alpha$ gene may be selectively inactivated in the hepatocytes using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse in particular alteration of the proliferation of the hepatocytes.

47. Transgenic mouse αAT-Cre-ER^{T2}/$RXR\alpha^{L2/L2}$ whose $RXR\alpha$ gene may be selectively inactivated in the hepatocytes using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse in particular alteration of the proliferation of the hepatocytes.

48. Transgenic mouse αAT-Cre-ER^{T3}/$RXR\alpha^{L2/L2}$ whose $RXR\alpha$ gene may be selectively inactivated in the hepatocytes using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse in particular alteration of the proliferation of the hepatocytes.

49. Transgenic mouse aP2-Cre-ER^{T}/$RXR\alpha^{L2/L2}$ whose $RXR\alpha$ gene may be selectively inactivated in the adipocytes

using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alteration of the metabolism of the lipids in the adipocytes and/or diabetes.

50. Transgenic mouse aP2-Cre-ER^{T2}/$RXR_{\alpha}^{L2/L2}$ whose RXR_{α} gene may be selectively inactivated in the adipocytes using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alteration of the metabolism of the lipids in the adipocytes and/or diabetes.

51. Transgenic mouse aP2-Cre-ER^{T3}/$RXR_{\alpha}^{L2/L2}$ whose RXR_{α} gene may be selectively inactivated in the adipocytes using a conditional deletion method following treatment with a synthetic ligand endowed with antiestrogenic activity, causing in said mouse alteration of the metabolism of the lipids in the adipocytes and/or diabetes.

52. Transgenic mouse according to one of Claims 40 to 51, characterized in that said RXR_{α} gene is inactivated by carrying out the method according to Claim 30.

53. Method of screening compounds capable of being used as a medicament for the preventive and/or curative treatment of alopecia and/or of hyperproliferation of the keratinocytes and/or of inflammatory reactions of the skin, characterized in that it comprises the step of administering said compound to a mouse according to Claims 40 to 45 and 52.

54. Method of screening compounds capable of being used as a medicament for promoting in particular hepatic regeneration, characterized in that it comprises the step of administering said compound to a mouse according to Claims 46 to 48 and 52.

55. Method of screening compounds capable of being used as a medicament for the preventive and/or curative treatment of diabetes and/or for the treatment of obesity, characterized in that it comprises the step of administering said compound to a mouse according to Claims 49 to 52.

56. Method of screening compounds capable of being used as a medicament for the preventive and/or curative treatment of skin cancers, characterized in that it comprises the step of administering said compound to a mouse according to Claims 40 to 45 and 52.

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